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Abnormal repetitive behaviors in zebrafish and their relevance to human brain disorders

Konstantin N. Zabegalov[a,b], Sergey L. Khatsko[b], Anton M. Lakstyga[c,d], Konstantin A. Demin[c,e], Madeleine Cleal[i], Barbara Dotto Fontana[i], Sebastian D. McBride[j], Brian H. Harvey[k], Murilo S. de Abreu[l], Matthew O. Parker[i] and Allan V. Kalueff[a,b,c,d,e,f,g,h*]

[a]School of Pharmacy, Southwest University, Chongqing, China

[b]Ural Federal University, Ekaterinburg, Russia

[c]Institute of Translational Biomedicine, St. Petersburg State University, St. Petersburg, Russia

[d]Granov Russian Scientific Center of Radiology and Surgical Technologies, Ministry of Healthcare, St. Petersburg, Russia

[e]Institute of Experimental Medicine, Almazov National Medical Research Centre, St. Petersburg, Russia

[f]The International Zebrafish Neuroscience Research Consortium (ZNRC), Slidell, LA, USA

[g]ZENEREI Research Center, Slidell, LA, USA

[h]Scientific Research Institute of Physiology and Basic Medicine, Novosibirsk, Russia

[i]Brain and Behaviour Laboratory, University of Portsmouth, Portsmouth, UK

[j]Aberystwyth University, Aberystwyth, UK

[k]Center of Excellence for Pharmaceutical Sciences, School of Pharmacy, Division of Pharmacology, North West University, Potchefstroom, South Africa

[l]University of Passo Fundo, Passo Fundo, Brazil

[*]**Corresponding author:**

Allan V. Kalueff, PhD, School of Pharmacy, Southwest University, Chongqing, China. Tel/Fax: +1-240-899-9571 E-mail: avkalueff@gmail.com

Abstract

Abnormal repetitive behaviors (ARBs) are a prominent symptom of numerous human brain disorders and are commonly seen in rodent models. As rodent studies of ARBs continue to dominate the field, mounting evidence suggests that zebrafish (*Danio rerio*) also display ARB-like phenotypes and may therefore be a novel model organism for ARB research. In addition to practical research advantages, zebrafish share high genetic and physiological homology to humans and rodents, including multiple ARB-related genes and stereotypic behaviors relevant to ARB. Here, we discuss a wide spectrum of stereotypic repetitive behaviors in zebrafish, data on their genetic and pharmacological modulation, and the overall translational relevance of fish ARBs to modeling human brain disorders. Overall, the zebrafish is rapidly emerging as a new promising model to study ARBs and their underlying mechanisms.

Keywords: zebrafish; abnormal repetitive behavior; stereotypy; animal models; human brain disorders

1. Introduction

Abnormal repetitive behaviors (ARBs) commonly occur in neuropsychiatric diseases, including obsessive-compulsive disorder (OCD), autism spectrum disorder (ASD), trichotillomania, Parkinson's disease, as well as Tourette's, Rett, Fragile X and Prader-Willi syndromes [1-3]. Typical ARBs include abnormal motor behavior, disrupted social interactions, aberrant goal-oriented behavior and self-injurious cycled actions [4]. In humans, the most frequent ARBs include skin-picking, head-hitting, repetitive manipulation of objects (spinning, twirling), repetitive use of language, body rocking, hand flapping, finger flicking and tics [5, 6]. Highly relevant clinically [1, 7, 8], some ARBs (e.g., skin-picking, hair-pulling) may also cause physical harm [9, 10]. Together, this emphasizes the growing clinical importance of ARBs and the need for their broad-scale translational research.

Animal experimental models are a powerful tool in neuroscience and biological psychiatry, markedly improving our understanding of CNS function and dysfunction [11-13]. Behaviorally, ARBs can be divided into two groups – motor stereotypies and impulsive/compulsive behaviors [14-16]. The former include the repetition of purposeless movements and/or body postures, whereas the latter involve cognitive inflexibility and aberrant goal-oriented behaviors [17-20]. Clinical motor stereotypies include repetitive stereotypical motor movements (SMMs), critical for neuropsychiatric diagnostics. Common SMMs include body rocking, hand flapping and finger moving, often seen in patients with ASD, Fragile X syndrome, Rett syndrome, Parkinson's disease (e.g., periodic fast/slow finger movements), and Huntington's disease [21-27]. Other common ARBs are tics, often occurring in Tourette's syndrome [28] as unconscious, abrupt, periodical and arhythmical movements or vocalizations [1, 29]. OCD symptoms include complex ARBs stemming from persistent recurrent compulsive ideas [30], combining composite behavioral acts (compulsions or rituals) with repeated behaviors (e.g., washing and cleaning) that, unlike tics, are conscious [1].

Given a wide spectrum of ARBs and multiple distinct CNS disorders with ARB-like phenotypes, the complex neurobiology of repetitive behaviors is poorly understood [31, 32]. However, the basic neuroanatomy and neuronal circuitry are beginning to unravel for some ARBs in both clinical and animal studies. For example, magnetic resonance imaging (MRI) in both humans and rodents has

revealed core brain structures involved in the regulation of motor behavioral patterns, including sensory motor and anterior cingulate cortex, cerebellum, thalamus and the basal ganglia [33-39]. Paralleling clinical findings, several rodent models with overt spontaneous stereotypies (e.g. deer mice, BTBR T+tf/J, C57BL/10, C57BL/6, C58 mice) are widely used to study ARBs, in which affected animals display repetitive jumping and self-grooming [40-43]. Neurochemical and clinical volumetric studies of the basal ganglia pathways implicate all major neurotransmitters in ARBs [44]. For example, OCD responds to selective serotonin reuptake inhibitors (SSRIs) [45], and disturbances in the serotonin transporter (SERT) are common in humans with OCD [46] and in animal models of this disorder [47, 48]. Likewise, gamma-aminobutyric acid (GABA), glutamate, noradrenaline, histamine, acetylcholine, cannabinoids, endogenous opioids and hypothalamic-pituitary-adrenal (HPA) axis hormones serve as reliable biomarkers of repetitive behavior [44, 49].

Various CNS disorders comorbid with ARBs have strong genetic determinants, including neuroligin (*NLGN*), GABA A-receptor β3 gene (*GABRB3*), methyl-CpG-binding protein 2 (*MeCP2*), the fragile X mental retardation (*FMR1*), contactin-associated protein-like 2 (*Cntnap2*), *SHANK* family, tuberous sclerosis complex 1 (*TSC1*), neurexin 1a *(NRXN1)* [50-52] and dopamine D3 receptor genes (*DRD3*) [53, 54]. Neuroligin genes (e.g., *NLGN3*) modulate dopaminergic signaling in ventral striatum [55], and mouse knockouts in *NLGN3* display robust motor stereotypies [55]. Other genes essential for GABA- and glutamatergic signaling are implicated in ARB pathogenesis [56]. For example, *MeCP2* (encoding transcriptional regulator MeCP2) and *GABRB3* (encoding the β3 subunit of the $GABA_A$ receptor) are associated with Rett and Prader-Willi syndromes [57-59]. Likewise, *GABARB3* knockout mice display repetitive circling and tail chasing [60, 61], whereas *MeCP2*-deficient mice exhibit impaired GABA signaling with forelimb stereotypies [62]. Genes related to aberrant glutamatergic signaling include *SHANK2* and other *SHANK* genes (responsible for stability of excitatory synapses [56]), and their disturbances may trigger repetitive jumping [63]. Mice lacking genes affecting glutamate NMDA receptors (e.g., *ninjurin 1/ning1*) and *grin1* (*glutamate ionotropic receptor NMDA type subunit 1*) exhibit compulsive grooming resembling clinical OCD [64, 65].

In summary, the genetic contribution to ARBs, established in preclinical and clinical genetics

studies (Table 1), confirm shared core mechanisms of ARB pathogenesis in humans and rodent models [66-68], calling for further translational research in this field. However, as humans and rodents share 80-85% genetic homology, it is logical to ask whether shared ARB pathways are generally evolutionarily conserved across vertebrate taxa? For example, while mutant mice with DAT genetic ablation show multiple repetitive behaviors [69-72], zebrafish *(Danio rerio)* with DAT genetic knockout can become a powerful model of DAT-mediated behavioral deficits. Generated recently, these mutant zebrafish display thigmotaxis (swimming closely to the walls of the tank, Fig. 3) [73] which may represent an ARB-like phenotype. Given a 70-75% of genetic homology between humans and zebrafish [74], their generally similar CNS [75] and core neurotransmitters, neurohormones [76], and their molecular targets [77-79], can experimental modeling of ARBs be extended to include fish models? In other words, can fish have ARBs? And, if they do, - how can ARBs of animals, separated from humans by thousands of years of evolution, inform us about core mechanisms underlying ARB pathogenesis?

2. ARB lessons from zebrafish

While the vast majority of pre-clinical ARB data have been obtained from rodent models [43, 80-86] (Table 1), the growing understanding of evolutionarily conserved core mechanisms of CNS disorders [12] necessitates novel models, new model organisms, and translational cross-species comparisons in the field of ARB research [87]. A small teleost fish, the zebrafish is rapidly gaining popularity in preclinical studies modeling human brain diseases [88] as a low-cost and research-efficient vertebrate organism [89] with fast development highly suitable for CNS research [90]. Notably, transparency of embryos allows the observation of zebrafish CNS *in vivo,* further enhanced by zebrafish brain using imaging tools [91]. Finally, remarkable genetic and physiological similarity to humans, simply quantifiable overt behavioral responses, shared neural circuits and sensitivity to psychotrophic drugs make zebrafish an appropriate model species in preclinical studies of human CNS disorders [91, 92].

Similar to rodent models, many basic behavioral patterns of zebrafish can be assessed in observation tanks similar to rodent open field tests, such as novel tank tests [93, 94]. Albeit not showing

some common rodent open field stereotypies (e.g., self-grooming), fish have their own set of stereotypic movements that can be recognized, quantified and modulated experimentally [95]. For example, zebrafish stereotypies are often observed in response to pharmacological intervention, and include repeated back-and-forth swimming at a particular part of the tank (e.g., at the bottom, middle, or top of the tank) [96], but may also include more specific behavioral pattern, such as stereotypic circling - repetitive round trajectory swimming, that is common for ketamine and other glutamatergic antagonists [97, 98] (Table 2). Furthermore, adult zebrafish may display repetitive thigmotaxis often seen following psychostimulant (e.g., nicotine) administration, and manifested as stereotypic swimming along the walls of the tank near the surface (similar to stereotypic locomotion of other model species in the open field test [99]).

However, it is premature to interpret such behavioral patterns without thorough mechanistic analyses and complementing behavioral observations with pharmacological and genetic challenges to target ARBs. For example, recent studies of the neurophysiological underpinnings of repetitive turning and other ARB-like behavior have focused on zebrafish larvae, revealing an important role of hindbrain in such fish phenotypes [100-102]. Likewise, assessing thigmotaxis and its relevance to ARBs in rodents [103] and zebrafish [99], such responses can be also related to alternation in luminance, and represent a tendency to swim outward (rather than the preference for the edges) [104].

2*.1. Autism-related models*

Like in rodents, disruption of some ASD-related genes provokes ARB-like phenotypes in zebrafish. For example, *SHANK3* knockout zebrafish display aberrant circling, thigmotaxis, corner-to-corner swimming and ‘looped’ figure-8 swimming [105]. With high homology of *SHANK3* between rodents and zebrafish (Table 2), such fish ARBs resemble stereotypies in mouse mutants of this gene [106-108]. *SYNGAP1* encoding synaptic Ras GTPase activating protein 1 is a critical regulator of glutamatergic NMDA-receptors [109] implicated in ASD [109, 110]. Zebrafish *SYNGAP1* knockouts display remarkable stereotypic movements, including prolonged undulating swimming with frequent C-bends, accompanied by aberrant mid- and hindbrain development [111]. Contactin-associated protein-like 2 gene (*CNTNAP2*) triggers epilepsy and ASD [112, 113] by disrupting inhibitory GABA-

ergic neurotransmission [114, 115]. In line with ASD-like ARBs in *CNTNAP2* knockout mice [116, 117], zebrafish *CNTNAP2* mutants also display higher responsivity to GABA-A receptor inhibitors, causing circling and burst-like movements [118]. Thus, the disruption of key ASD-related genes leads to the development of stereotypic movements in zebrafish.

The fragile X syndrome, clinically distinct from ASD, has an overlapping ARB phenotype with typical stereotypic movements, hand flapping and biting [119-121] triggered by aberrant activity of an X chromosome gene *FMR1* (fragile X mental retardation 1) crucial for CNS development, neurotransmission and synaptic stability [122, 123]. *FMR1* knockout rodents display aberrant jumping, circling, digging and increased self-grooming [124, 125]. Although zebrafish *FMR1* knockouts and knockdowns have craniofacial alterations, aberrant neurotransmission (e.g., cholinergic in motor neurons, glutamatergic in the CNS) and behavioral changes (e.g., hyperactivity [126-129]), their ARBs have not yet been noted [127], necessitating further studies using this model organism.

Rett syndrome is another debilitating disorder genetically related to the X chromosome, mainly affecting females and manifesting in stereotyped hand wringing, rubbing or clapping movements [130]. The main candidate gene for Rett syndrome is *MeCP2* [131], and *MeCP2* knockout mice display similar neurological deficits [132], including ARB-like hindlimb clasping and altered dopamine and glutamate signaling [133, 134]. Zebrafish *MeCP2* knockouts display abnormal thigmotaxis, likely associated with neurodevelopmental abnormalities in the hindbrain [135], and the *MeCP2* knockdown impairs neurodevelopment and neurodifferentiation in larval fish [136].

2.2. Modeling obsessive-compulsive disorder in zebrafish

There are strong parallels between OCD and other ARB-related conditions, and animal models of OCD proposed based on their phenotypic stereotypy profiles, include genetic models (e.g., hyper-dopaminergic mutant deer mice [137, 138] and *Sapap*, *Slitrk5* and *HoxB8* knockout mice [139]), drug-induced and some other models [43, 140, 141]. Zebrafish models of OCD are gaining value in neuropsychiatric research [142-145]. Currently, there are many behavioral tasks that can be used to assess OCD phenotypes in zebrafish and that may be differently classified when using larvae or adult animals. Larvae OCD-like phenotypes are commonly analyzed by video-tracking software and

comprise subtle stereotypic movements such as dashing, freezing and repetitive rotational turns [142]. A recent method to analyze swimming behavior in zebrafish larvae [146] improves the analysis of their behavioral profiles and can be used as an important tool for OCD drug discovery assays. In addition to larvae, OCD phenotypes can be measured in adult zebrafish by assessing their stereotypic movements and compulsive choice [93, 142] (see further).

The early studies using adult zebrafish focused on drug-induced locomotor effects to better understand OCD-related stereotypic behavior in zebrafish novel tank test, a paradigm similar to the open field in rodents [93]. For example, this has revealed stereotypic behavior in adult zebrafish expressed as repetitive rotations or "circling behavior", such as those induced by NMDA receptor antagonists (e.g., ketamine) [147]. This approach has clear translational concordance with OCD and its treatment [148, 149], as ketamine and other NMDA antagonists often evoke stereotypic circling in humans and rodents. Zebrafish exposed to ibogaine (a hallucinogen with some NMDA antagonist activity that induces stereotypic behavior in rodents [150]) display circling behavior and repetitive corner-to-corner swimming [151]. Repetitive, unvarying perseverative behavior without goal or function has been described in zebrafish following cocaine withdrawal [130]. The predictive validity of stereotypic behavior in translational models is based on response to SSRIs which ameliorate OCD symptoms [152]. Notably, 5-HT1B receptor antagonists can induce repetitive behavior in zebrafish [153] that can be reversed by known OCD treatments (e.g., fluoxetine [154]) with striatal activation modulated only by specific OCD treatments [153].

Compulsive choice is another important OCD-related behavior frequently studied in rodent models [69] by subjecting the rodent to the spontaneous alternation test [155] and using the "signal attenuation" model [82]. In zebrafish, a compulsive choice can be studied in a T- or Y-maze assessing habit formation. Briefly, during acquisition of a learning task, normal animals will use both olfactory and visual stimuli to learn the location of food. Once the task is well learned, the animal will develop a 'habit' in which the amount of cognitive processing of the array of stimuli in the environment will be lower, as evidenced by lower sensitivity to devaluation, and by reduced sensitivity to contingency degradation [156-158]. Importantly, alterations in habit-forming have been observed in OCD patients

[159], suggesting that such behavior is an important marker of this disorder [160, 161]. In line with this, zebrafish exposed to alcohol during early brain development form habits early in the learning process in an adaptation of the T-maze "place-response" test Parker, Evans [162]. Such tests can be further validated by drugs traditionally used to treat OCD-related symptoms (e.g., fluoxetine), thus providing face and predictive validity for zebrafish models of stereotypic behavior and habit formation in OCD-like phenotypes [93, 142].

2.3. Cognitive inflexibility

Cognitive flexibility is the ability to adjust and adapt cognitive processing strategies in response to new, unexpected challenges [163]. Conceptually, it is the opposite end of the spectrum to ARBs, which are rigid and fixed. Therefore, understanding the biology of cognitive and behavioral flexibility may offer much to the study of ARBs, and vice versa [85]. Thus, to more closely translate the animal model to human OCD [1], assessment of cognitive flexibility-rigidity needs to be related to observed compulsive behaviors [164]. One method of measuring behavioral flexibility is attention set shifting tasks, which requires learning the response to a simple 'rule' applied to a complex stimulus, to identify relevant or non-relevant cues, and then modifying the response when the rule is changed, i.e. responding to the previously irrelevant (instead of the relevant, reinforced) cue [165]. Reductions in cognitive flexibility are seen in patients suffering from various neuropsychiatric disorders, including OCD and ASD [166], making it an important endophenotype to observe and model [167, 168].

Many neuropsychiatric diseases affecting the frontal cortex have deficits in cognitive flexibility signified by increased perseveration for the previous rule and increased errors shifting from one rule to the next [166]. Notably, the severity of the condition (i.e., in OCD patients) correlates with the deficit in reversal learning [166, 169]. Another paradigm, similar to that in primates and rodents [165, 170], has been adapted for zebrafish. For example, zebrafish are able to discriminate two colored cues (using a food reinforcer), demonstrating the capacity for to make 'choices' about differently valued stimuli. Zebrafish are also capable of cognitive flexibility, in terms of their responses to reversal learning and intra-dimensional set-shifting [171]. During a typical reversal learning protocol, an animal initially is trained (Phase 1) on a discrete-trial protocol to discriminate between two

differentially reinforced stimuli (e.g., colors: RED = *S*+ [reinforced], GREEN = *S*- [non-reinforced]). Once it has reached a criterion of response allocation to the reinforced alternative (e.g., 6-correct responses in a row), the reinforced and non-reinforced alternatives are reversed (GREEN = *S*+, RED = *S*-; Phase 2). During Phase 2, the animal initially shows low correct responses, but gradually learns that *S*- from phase 1 is now *S*+, and reaches criterion on Phase 2. In Phase 3, the colors are switched to a new pair (intra-dimensional set shift; e.g., BLUE = *S*+, YELLOW = *S*-). In the final phase (Phase 4), the two new colors are reversed. If the animal is showing cognitive flexibility, the hypothesis in a reversal learning experiment such as this is that the animal will reach criteria more quickly as the phases continue, on account of their switching the 'rule' by which they are performing responses on the task in an adaptive manner. Zebrafish require progressively fewer trials to reach learning criterion as a function of phase, confirming that this species can be cognitively flexible [172]. Thus, zebrafish performance on tasks of cognitive flexibility renders them ideal for the study of ARB, as cognitive inflexibility is a hallmark of ARBs. Together with the ease of genetic and pharmacological manipulations, zebrafish may further our knowledge on the cognitive-psychobiological aspects of cognitive flexibility in ARB-related disorders.

Another area of executive function that can be measured is working (e.g., spatial) memory [173]. The Y-maze (Fig. 4), a three-armed maze to record spontaneous alternation [174], has been adapted for zebrafish [175] as a useful tool for testing fish. Mazes can be set up in the presence or absence of any motivational or emotional factors, therefore permitting measures of motivation and learning or pure novelty seeking with minimal confound [175]. Automation of this task has enabled minimum user interaction and ease of recording several different variables from a single trial. A recent study employing the Y-maze used an analysis of overlapping tetragrams (i.e., in 100 trials, 16 overlapping tetragrams ranging from RRRR to LLLL [176]) to determine how zebrafish explore the maze in a 1-h trial, revealing aberrant alternations in fish developmentally exposed to ethanol. Thus, the Y-maze has the potential of a flexible and relatively high-throughput method for assessing executive functions associated with learning and working memory. With some further investigation, the Y-maze can be an excellent tool for evaluating neuropsychiatric disorders with both extreme and

more subtle ARBs, thus broadening our ability to model cognitive dysfunction in zebrafish. Indeed, age-related changes in patterns of alternation and repetition have been found in zebrafish (Fig. 4). Given overt stereotypies as part of the typical behavioral repertoire of infant humans (reducing with age in normative development) [177, 178], repetitive movements in the aquatic Y-maze may mimic ARBs observed in human development.

3. Existing challenges, model limitations, and future directions

Clearly, numerous challenges exist in the development of zebrafish models of ARBs. For example, how to properly translate animal repetitive behavors into human ARBs? Indeed, several of clinical ARB symptoms are difficult, if not impossible, to observe in zebrafish. Therefore, the question is whether the behaviors selected in order to determine ARB are sufficient to call these animal behaviors ARBs. Another problem concerns the overall reliability of behavioral tests recognized recently and requiring an urgent sonution [179, 180]. One way to solve it is to ensure that standard protocols are published and utilised by groups using the same behavioral endpoints. Another strategy is to ensure automation is used as widely as possible. As stated earlier, this will be expedited by the recent advent and availability of commercially available automated testing hardware. Third, laboratories should adhere to standardized reporting protocols, such as the ARRIVE guidelines [181], to ensure that intra-laboratory procedures are transparent and fully repeatable, aiming to maximize interlaboratory reliability. Fourth, laboratories should be encouraged to share data and protocols in a timely manner, even from negative experiments, via preprint online servers, to enable fast and accurate reproduction of protocols across the community, and facilitate interlaboratory collaboration, if necessary.

In addition to challenges mentioned above, one of the most useful aspects of the zebrafish model is the ability to carry out high-throughput testing in a vertebrate system. To a certain extent, this is possible in adult fish using protocols outlined above. However, there are some drawbacks to using adult zebrafish which are similar to those associated with mammalian model systems, includung practical problems with cost of housing, space constraints, long-term isolation of a sentient social species, and individual behavioral variance. Therefore, the more active use of larvae should be

considered, especially given the development of larval assays of complex behaviors (e.g., impulsivity) [182] which may be usful in the early characterization of ARBs, with strong links between behavioral compulsions and impulse control [183, 184]. Finally, in order for zebrafish to prove useful to study mechanisms of ARBs, several fundamental questions need to be addressed. Indeed, the underlying mechanics of normal action selection in zebrafish remain unclear. For example, what neural circuits underlie choice behavior, behavioral flexibility, and balance between various basal ganglia pathways? Once we have the answers to this question, zebrafish will be extremely useful in understanding the neural circuits underlying ARB (also see Table 2 for strategic directions in the study of ARBs using this organism).

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Figure 1. Selected repetitive behavior in zebrafish. Panel A shows how drugs can affect zebrafish behavior in novel tank test. For example, acute ketamine exposure may induce characteristic repetitive behavioral patterns in zebrafish swimming, paralleling ketamine-evoked circling in rodents and clinical stereotypies (see [147] for details). Panel B illustrates thigmotaxis in adult zebrafish, as they typically prefer to swim close to the walls of the tank [185]. Albeit potentially reflecting increased anxiety-like behavior in some contexts (e.g., anxiogenic center avoidance), this response may also represent a pathological repetitive behavior (e.g., evoked by psychostimulants, such as nicotine) relevant to stereotypic peripheral hyperlocomotion, commonly seen in rodents (e.g., following psychostimulant drugs) (adapted from [186]).

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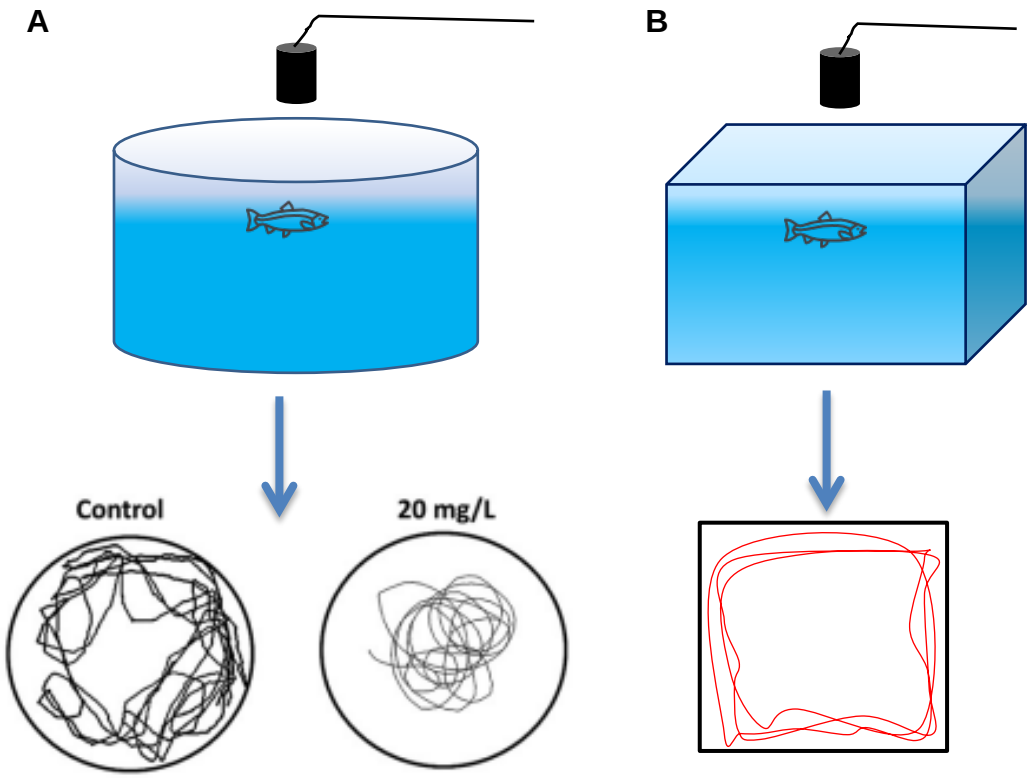

Figure 2. Selected examples of genetic models of aberrant repetitive behaviors in zebrafish. SHANK3 (left panel) is an autism-related gene that encodes postsynaptic density protein (PSD, binding to glutamatergic NMDA receptors) whose ablation in mice impairs synaptic transmission. Knockdown of SHANK in zebrafish up-regulates NMDA receptor and evokes ARB-like repetitive circling, corner-to-corner and figure-8 swimming (top view), according to [105]. A synaptic ras GTPase-activating protein SYNGAP1 (right panel) is another key protein involved in synaptic transmission, whose hypofunction in mice induces precocious maturation of synapses and increases synaptic transmission. NMDA receptor interact with postsynaptic density-95 (PSD-95) protein, which binds to SYNGAP. SYNGAP1 knockdown zebrafish demonstrate overt stereotypic movements, including prolonged undulating swimming with frequent C-turns (top view), according to [187]

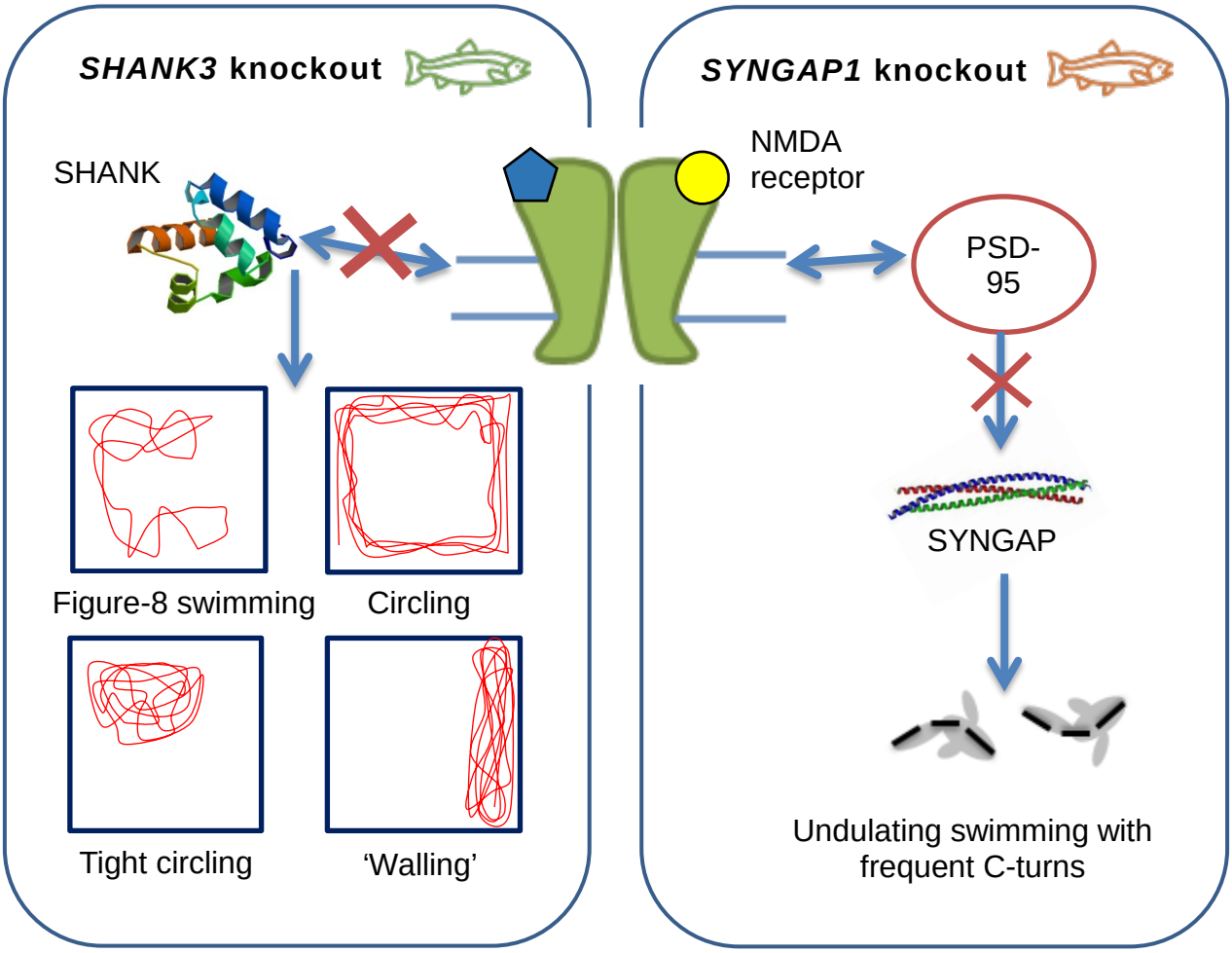

Figure 3. A brief summary of ARB-like behavioral phenotype of the dopamine transporter (DAT) knockout zebrafish, including swimming predominantly at the bottom of the tank with characteristic thigmotaxis (moving along the walls of the tank), according to [73]

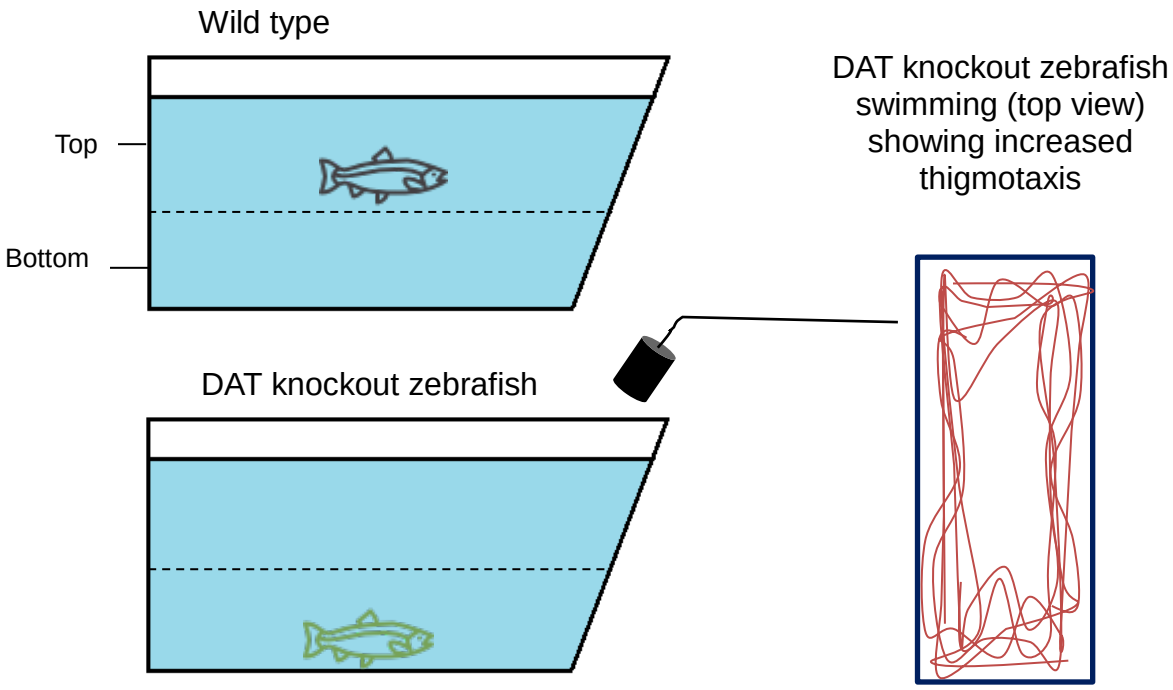

Figure 4. The use of Y-maze to assess behaviorally flexible patterns of swimming (alternation and repetition) in zebrafish. This test also reveals certain developmental changes in zebrafish swimming, ranging from pure alternation (LRLR, RLRL) to pure repetition of previous response (RRRR, LLLL, Parker laboratory, unpublished data). Overall, young fish show high levels of pure repetition and pure alternation, whereas older zebrafish show lower levels of repetition relative to alternation.

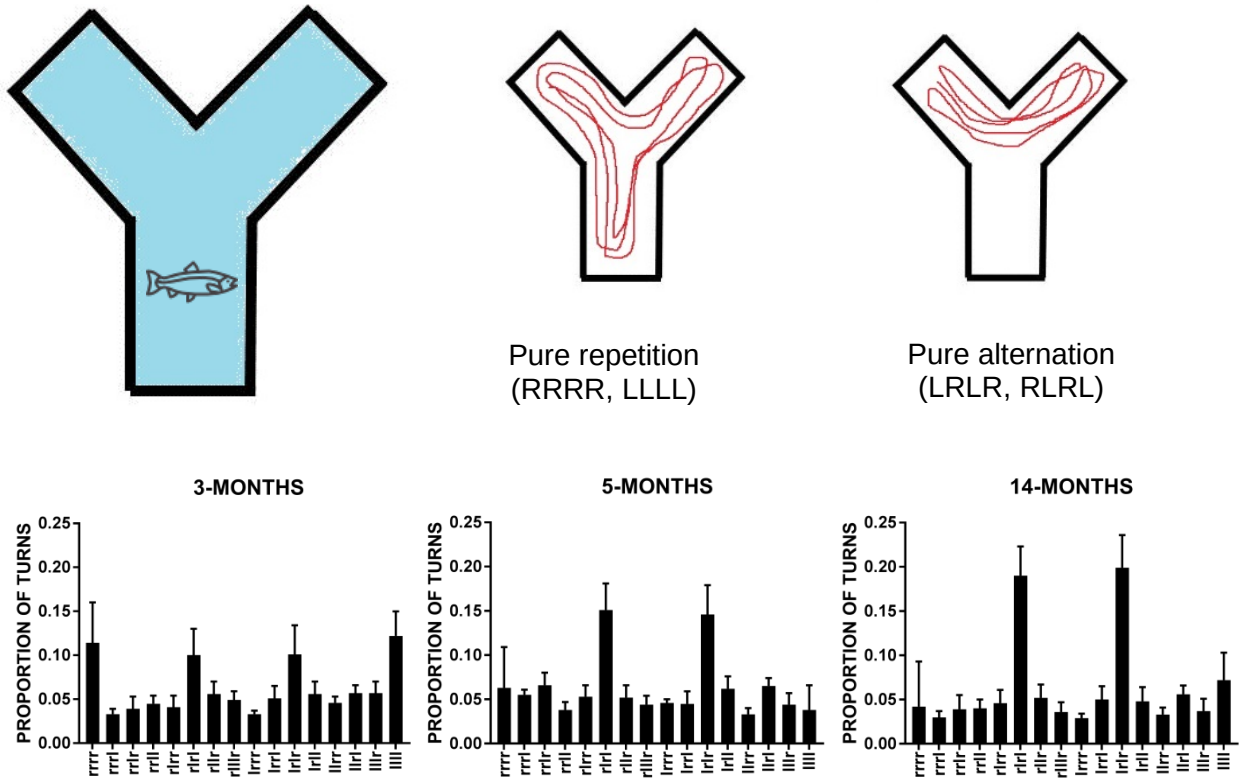

Table 1. Selected animal models that parallel clinical symptoms of ARBs

Rodents		**Zebrafish**	
ARB	**References**	**ARB**	**References**
Pharmacological			
Rat ASD model (prenatal valproate) evokes repetitive locomotion (back-and-forth moving)	[188]	Acute ketamine* induces increased circling behavior, Fig. 1	[147]
Amphetamine exposure in C58/J mice evokes repeated cage-lid back-flipping	[65]	Acute dizocilpine (MK-801)* increased circling behavior	[98]
Rat 6-OHDA** brain lesions (a Parkinson's model) evoke compulsive lever-pressing under chronic pramipexole***	[189]	Phencyclidine (PCP)* increased circling behavior	[190]
Rat prenatal exposure to lipopolysaccharide (LPS) increases repetitive self-grooming	[191]	The mixture of crude oil with lead increases cycle swimming in 15 larvae	[192]
Deer mice exhibit increased repetitive jumping following apomorphine***	[193]	Stereotypic corner-to-corner swimming at the bottom of the tank under ibogaine	[151]
Genetic			
Shank1 knockout mice display increased self-grooming	[194]	Adult *mecp2* mutants exhibit overt thigmotaxis	[135]
Histidine decarboxylase knockout mice display increased self-grooming	[195]	Shank3b knockouts display figure "8" swimming, circling, cornering and walling (Fig. 2)	[105]
Mice with deleted *Netrin-G ligand 2 (NGL-2)* gene display increased self-grooming	[196]	*Syngap1a* knockdowns escape responses with prolonged repetitive C-bends	[111]
Hoxb8 KO mice (an OCD model) display pathological self-grooming	[139]	*CNTNAP2* mutant larvae display burst-like and circling movements	[118]
MeCP2 deficient mice display stereotyped forepaw movements and compulsive self-grooming	[62]	Adult DAT knockouts exhibit increased thigmotaxis	[73]

*An antagonist of glutamate NMDA receptors
**6-hydroxydopamine, a neurotoxic antidopaminergic agent
***An agonist of several dopamine receptors

Table 2. Comparative genetic homology between human, rodent and zebrafish ARB-related genes, based on the Basic Local Alignment Search Tool (BLAST, www.blast.ncbi.nlm.nih.gov) database

Gene	Comparison: query coverage/homology		
	Human vs Mouse	Human vs Zebrafish	Mouse vs Zebrafish
SLC6A3	48%/87%	42%/79%	48%/78%
FMR1	96%/89%	30%/75%	30%/75%
MeCP2	82%/82%	5%/70%	5%/68%
CNTNAP2	64%/81%	38%/68%	54%/68%
SHANK1	73%/85%	28%/70%	35%/72%
SHANK2 (zebrafish – shank2b gene)	48%/86%	35%/70%	27%/76%
SHANK3 *	99%/85%	34-41%/71%	35-41%/73%
TSC1 (zebrafish – tsc1a, tsc1b)	64%/81%	7-17%/69-72%	8-23%/69-73%
*GABRB3***	97%/81%	20%/79%	21%/80%
DRD3	86%/88%	44%/74%	51%/75%
5-HT2C	96%/83%	15%/70%	16%/69%
*SYNGAP1****	67%/ 92%	43%/73%*****	No similarity
HOXb8 (zebrafish: hoxb8a, hoxb8b)	99%/90%	37%/71-75%	25%/70-75%
SLC1A1	82%/81%	37%/70%	38%/72%

* Zebrafish shank3a – PREDICTED transcript variant X18, shank3b – PREDICTED transcript variant X4
** Human/mouse – transcript variant 1, zebrafish – PREDICTED transcript variant X1
*** Human – transcript variant 1, zebrafish: syngap1a – PREDICTED transcript variant X2, syngap1b
**** No similarity with syngap1b

Table 3. Selected open questions in the field of zebrafish modeling of ARBs

Questions
Conceptual
• What is entire spectrum of neurobehavioral ARB-like phenotypes in zebrafish?
• Which brain structures are implicated in zebrafish ARBs?
• Are there links between zebrafish ARB and social behavioral deficit?
• How is normal motor sequence selection modified (varied) based on prior motor sequence performance in zebrafish?
• How do alterations in mechanisms of motor sequence selection lead to repeated invariant behavioral sequences (ARBs)?
• Are ARBs a neurological phenomenon, or the result of alterations in interaction with the environment?
• Can ARBs spontaneously emerge in zebrafish (i.e., as a result of chronic isolation/under-stimulation)?
• Can a zebrafish be 'bored'?
• Can ARBs in zebrafish be qualitatively differentiated from 'normal' behavior, and modeled, mathematically?
• Do larval zebrafish display overt ARBs? Are they similar to those seen in adult fish?
• Is there a pathological link between ARBs, self-aggression, and aggression? Can this be modeled in zebrafish?
• Do ARBs display aging-related trajectories in zebrafish?
• How does stress affect zebrafish ARBs?
• Do zebrafish use ARBs in social or sexual contexts?
• Do zebrafish ARBs display circadian rhythms?
Translational
• What are the mechanisms of normal motor sequence selection and invigoration in zebrafish?
• What is the homology in mechanics and/or circuitry of motor sequence selection between zebrafish and mammals?
• If zebrafish ARB can be quantified into subunits or predictable patterns? Can they help test drugs or mimic human ARB?
• Is there a substantial homology between human and zebrafish ARB-related neurocircuitry?
• Do stress-evoked alterations in zebrafish ARBs resemble those evoked in human ARBs?
• Do zebrafish ARBs respond to various drugs similarly to human ARBs?
• How does impulsivity contribute to zebrafish ARB expression?
• How does zebrafish individuality ('personality') affect ARB-like phenotypic variance in zebrafish populations?
• Are there robust sex differences in some zebrafish ARBs similar to those in humans with certain CNS disorders?
• Are there common/shared epigenetic mechanisms of ARB regulation in mammals and zebrafish?
• Do aging-related ARBs in zebrafish resemble those observed in aging humans?
Methodological
• Can zebrafish ARBs be fractionated into quantifiable sub-units, in terms of predictable patterns of expression?
• What is the potential for the development of a zebrafish ARB ethogram?
• Can a zebrafish be trained to produce ARB?
• Can a zebrafish that shows ARB be trained to stop producing these patterns?
• What are neuroendocrine biomarkers of zebrafish ARBs?
• Are there well-established strain differences in zebrafish ARBs?
• Do zebrafish ARBs differ between the laboratories and/or between different vendors?
• Do wild-caught zebrafish display ARBs? Do ARBs increase during domestication?
• To what extent ARBs may concomitantly affect other neurobehavioral responses
• Are there reliable tools for automated quantification of ARBs in zebrafish?
• Are tools available for high-throughput multi-animal detection of ARBs in zebrafish groups?
Others
• Do zebrafish ARBs represent an animal welfare problem?
• Can improved welfare (e.g., by using environmental enrichment) reduce zebrafish ARBs?

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